Thiamine Analogues featuring Amino-oxetanes as Potent and Selective Inhibitors of Pyruvate Dehydrogenase

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Abstract

Pyruvate dehydrogenase complex (PDHc) is suppressed in some cancer types but overexpressed in others. To understand its contrasting oncogenic roles, there is a need for selective PDHc inhibitors. Its E1-subunit (PDH E1) is a thiamine pyrophosphate (TPP)-dependent enzyme and catalyses the first and rate-limiting step of the complex. In a recent study, we reported a series of ester-based thiamine analogues as selective TPP-competitive PDH E1 inhibitors with low nanomolar affinity. However, when the ester linker was replaced with an amide for stability reasons, the binding affinity was significantly reduced. In this study, we show that an amino-oxetane bioisostere of the amide improves the affinity and maintains stability towards esterase-catalysed hydrolysis.

Pyruvate dehydrogenase complex (PDHc) links glycolysis and the Krebs cycle by catalysing the conversion of pyruvate into acetyl coenzyme A (acetyl CoA).[1-4] Its E1-subunit (PDH E1) is a thiamine pyrophosphate (TPP)-dependent enzyme and catalyses the first and rate-limiting step of the complex. Due to its key role in cellular bioenergetics, the activity of PDHc is tightly regulated: phosphorylation of PDH E1 by PDH kinases (PDKs) deactivates the complex, whereas dephosphorylation by PDH phosphatase activates it. Many types of cancer cell increase the expression of PDKs, suppressing PDHc activity, so inhibition of PDK has been an anticancer strategy.[4-6] However recent findings have shown that certain cancers overexpress PDHc,[7-9] and an irreversible inhibitor of PDH E1 suppresses development of one of these types of cancer in a mouse model.[9] The contrasting evidence on the role of PDHc in oncology implies that the effect of PDHc modulation depends on the type of tumour and its environment.[7-9] One approach to unveiling its puzzling role is through chemical inhibition of PDH E1.

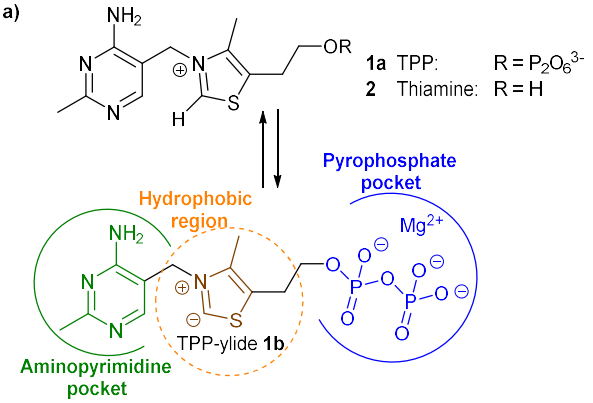
TPP **1a** is the bioactive form of thiamine (vitamin B_1) **2**.[1-3] TPP-dependent enzymes encompass diverse activities and include PDH E1, pyruvate decarboxylase (PDC), pyruvate oxidase (PO), 1-deoxy-D-xylulose 5-phosphate synthase (DXPS) and 2-oxoglutarate dehydrogenase E1-subunit (OGDH E1). The architecture of the coenzyme-binding pocket is highly conserved across the enzyme family and favours formation of the catalytically active ylide **1b** (Figure 1a) by placing the thiazolium ring in a hydrophobic environment.[1-3] A common strategy in designing inhibitors of the TPP-dependent enzyme family lies in the use of thiamine/TPP analogues.[10-21] These analogues typically feature a neutral central aromatic ring to replace the positively charged thiazolium ring, *e.g.* thiophene,[10] triazole,[11] pyrazole,[12] furan[13] or pyrrole[18]. This neutral ring mimics the thiazolium ylide **1b** and so captures the strong stabilising interactions between the enzyme and the high-energy ylide.[21]

Our group have developed thiamine/TPP analogues to study the enzymology[10,21] and the cellular roles[19,20] of this protein family. Recently, we reported ester **3** as a TPP-competitive inhibitor of porcine PDH E1 (Figure 1b).[17] The aryl ester tail makes **3** a selective inhibitor of mammalian PDH E1 as other TPP-dependent enzymes have a smaller pyrophosphate pocket that cannot accommodate the ester. In another study,[19] the ester linker of **3** was replaced by an amide **4** to improve its intracellular stability.

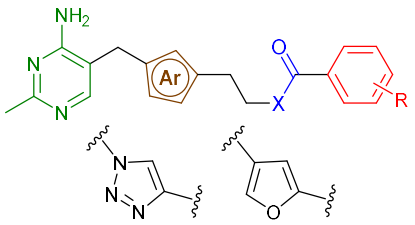
However, this resulted in six times weaker inhibition. Changing the scaffold from triazole **3** to furan **5** improved binding and furan amide **6** had improved chemical stability and maintained binding affinity relative to triazole ester **3**. In further structure-activity relationship (SAR) studies, we identified that a *m*-pyridin-3-yl substituent (**7**) on the benzoyl terminus led to better binding and amide-linked analogue **8** exhibited micromolar cytostatic effects on several cancer cell lines.[19]

While amide **8** is a useful chemical probe for inhibition of intracellular mammalian PDH E1,[19] it could be improved. First, its nanomolar K_I value towards PDH E1 translated into merely micromolar cytostatic activity in cell-based assays. This is possibly because it is competitive with TPP and its affinity for PDH E1 is only four times greater than that of TPP, and so cells may accumulate slightly more TPP to outcompete the inhibitor.[19] Second, its terminal *m*-pyridin-3-yl substituent may exhibit undesired metabolic instability and/or toxicological effects (due to the exposed nitrogen atom).[22] Third, its accessibility is limited by the long synthetic route - twelve chemical reactions were involved.[19] In this work, by incorporating recently reported synthetic methodology for amino-oxetanes,[23] we developed novel thiamine analogues that are not only selective and stable, but also more potent and synthetically accessible than amide **8**.

a)



b)



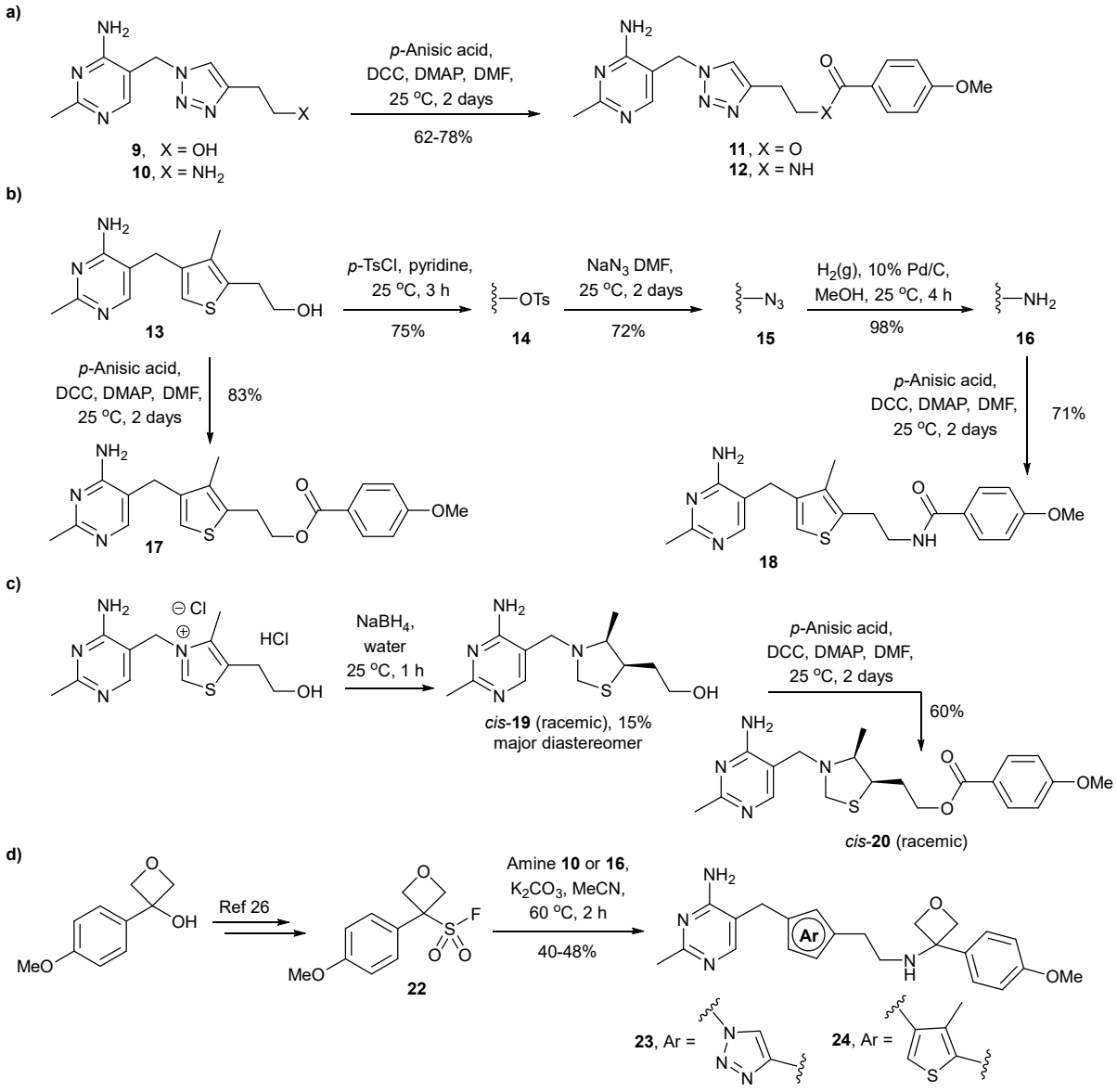
Compound	Ar	X	R	IC_{50} (μM)
3	Triazole	O	*p*-chloro	10.6
4	Triazole	NH	*p*-chloro	66.0
5	Furan	O	*p*-chloro	3.3
6	Furan	NH	*p*-chloro	9.2
7	Triazole	O	*m*-pyridin-3-yl	3.0
8	Furan	NH	*m*-pyridin-3-yl	2.5

Figure 1. a) Structure of thiamine **2** and its bioactive form, TPP **1a**. **b)** Inhibitory activities on PDH E1 of selected thiamine analogues **3-8**[17,19] (IC_{50} data were determined at [TPP] = 10 μM).

In the current work, the aminopyrimidine and phenyl moieties of **3**-**8** were kept unmodified so as to retain target affinity and selectivity. The terminal *m*-pyridin-3-yl group of **7**-**8** was replaced because it may lead to off-target effects as a result of cytochrome P450 inhibition[22] and its group efficiency (GE, kcal mol^{-1} of binding energy per heavy atom) was not that high (0.21, where >0.3 is preferred, Figure S1).[24,25] Docking models predicted that the nitrogen atom of the pyridine ring hydrogen-bonds to the protein.[19] Here we employed a structure-based ligand design strategy to identify small substituents (with 1-2 heavy atoms) capable of forming hydrogen bond(s) in the pyrophosphate pocket. After rounds of docking, a *p*-methoxy group was selected (Figure S2). To synthesise the selected compounds, triazole-based alcohol **9** and amine **10** were coupled to *p*-anisic acid to give the corresponding ester **11**[26] and amide **12** (Scheme 1a), using the same method as for **3** and **4**.[19]

Our studies on **3** to **8** showed that switching from triazole to furan improves binding.[19] Subsequently, we systematically evaluated a range of thiamine/TPP analogues featuring different aromatic central scaffolds.[21] Among the four tested five-membered aromatic rings, the more hydrophobic central rings (reflected by their cLog*P* values) resulted in stronger inhibition (affinity: thiophene > pyrrole > furan > triazole). This trend was observed not only on PDH E1, but also on other TPP-dependent enzymes.[21] Thus, thiophene was chosen in this study; the synthesis of thiophene alcohol **13** has been described

before[21] and its conversion into ester **17** and amide **18** is shown in Scheme 1b. Most reported thiamine analogues feature a planar aromatic ring[10-21] as the central scaffold. To assess whether there is any advantage of including a non-planar ring, a saturated thiazolidine ring, tetrahydrothiamine **19**, was included. Sodium borohydride reduced the central ring of thiamine hydrochloride, yielding thiazolidine **19** (Scheme 1c) as a mixture of two racemic diastereomers with *cis*-**19** being the major product (2.5:1 ratio).[27] After two rounds of flash chromatography, we obtained racemic *cis*-**19** containing less than 10% of trans-**19**. Ester *cis*-**20** was prepared from *cis*-**19** (Scheme 1c), but the corresponding amide could not be obtained by our standard method. The tosylation step was unsuccessful, and we suspect that the nitrogen atom of the cyclic amine might have cyclised with S_N2 displacement of the tosylate.



Scheme 1. Synthesis of esters **11**, **17** and **20**, amides **12** and **18**, and amino-oxetanes **23** and **24**.

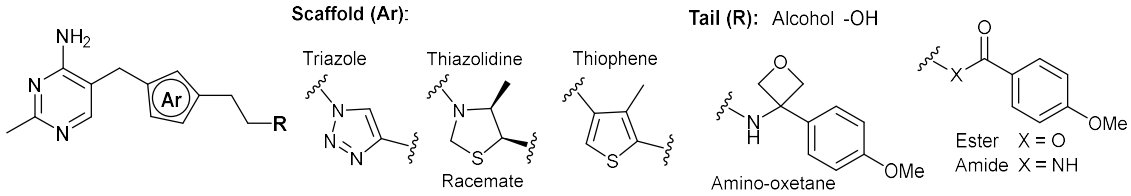
Previously, we replaced the ester linker of our thiamine analogues with an amide (Figure 1b) for better intracellular stability but this caused a drop in affinity.[19] Thus, a key goal here was to identify a suitable amide bioisostere that binds more strongly than the amide but remains resistant to (enzymatic) hydrolysis. A recent review provided a comprehensive overview of amide bioisosteres[28] and from this we chose amino-oxetane due to its preferred physiochemical properties and its predicted binding

interactions; Table S1 gives an overview of how we made this choice. Synthesis of an N-alkyl-N-oxetan-3-ylamine could in principle be accomplished either by formation of the N-alkyl bond, *e.g.* by N-alkylation of the primary oxetan-3-ylamine[28-29] or by reductive amination,[29] or by formation of the N-oxetane bond. As we already had the amines **10** and **16** in hand, we preferred the latter. Fortunately, the Bull group have recently reported a novel method to form amino-oxetanes through coupling of an fluorosulfonyl-oxetane **22** with amines in an amide bond-like disconnection.[23] Using this method, amines **10** and **16** were converted into amino-oxetanes **23** and **24** (Scheme 1d).

The potencies of the synthesised compounds as inhibitors of PDH E1 were evaluated on commercially available porcine PDH E1 which is a widely accepted substitute for human PDH E1.[14,18,19] The inhibitory activities are summarised in Table 1 and Figure S7. Regarding the terminal substituent, although **11** was not quite as potent as **7** (IC_{50}: 6.3 *vs.* 3.0 µM), the *p*-methoxy group does contribute to binding (**11** > **3**, Figure 1b). More importantly, compared to the *m*-pyridin-3-yl group (**7**), the *p*-methoxy group (**11**) binds more efficiently (GE: 0.39 *vs.* 0.21, refer to Figure S1 for calculations) and does not possess an exposed nitrogen atom which may lead to undesired pharmacological effects. As summarised in Table 1, the potency of the central ring was triazole < thiazolidine < thiophene regardless of the tail group (**9** < *cis*-**19** < **13** and **11** < *cis*-**20** < **17**). The cLogD values at pH 7.4 of the heterocycle with just methyl substituents were estimated (using MarvinSketch 21.2, Figure S8) as -0.14, 1.52 and 3.27, respectively (none of the three heterocycles are expected to be protonated at neutral pH). The exit vector geometry of the saturated thiazolidine ring is likely to be somewhat different from that of the two planar aromatic rings;[21,25] but despite this, the relative potency of these rings mirrored their relative hydrophobicity. The thiazolidine *cis*-**20** was evaluated as a racemate so, if one enantiomer was more potent, it might be up to two-fold more potent than the racemate.[30] However, as **13** and **17** were about six- and four-fold more potent than racemic cis-**19** and cis-**20**, respectively, the most hydrophobic thiophene remains the best scaffold among the three, consistent with our earlier findings.[21] As shown in Table 1, changing the amide linker to an amino-oxetane restored affinity to a level almost comparable to the equivalent ester (**12** << **23** < **11** and **18** << **24** < **17**). Within the series, the thiophene-based ester **17** and amino-oxetane **24** were the strongest PDH E1 inhibitors, with K_I values in the single-digit nanomolar range.

The inhibitory action of some selected compounds was further evaluated biochemically in terms of their modality and selectivity. Their TPP-competitive relationship was confirmed as the observed potency decreased with increasing [TPP] (Table 1). Notably, only thiophenes **17** and **24** retained over 50% inhibition of PDH E1 in the presence of a five-fold excess of TPP. To assess their selectivity, the six compounds were tested on four other TPP-dependent enzymes (PDC, PO, DXPS and OGDH) at concentrations either equal to [TPP] or five-fold higher than [TPP]. No inhibition (<10%) was observed on any of these off-target enzymes.

Table 1. Summary of inhibitory activity on PDH E1.



Compound			PDH E1 Inhibition[a]					PAMPA
	Scaffold (Ar)	Tail (R)	% Inhib. (1:5)[b]	% Inhib. (1:1)[c]	IC_{50} [d] (µM ± SEM)	vs TPP[e]	K_I (nM)[f]	% absorbed
9	Triazole	-OH			30[g]	0.33	150[g]	
cis-**19**	Thiazolidine	-OH			21 ± 4	0.48	105	
13	Thiophene	-OH			3.6[g]	2.78	18[g]	
11	Triazole	Ester	25 ± 4	63 ± 3	6.3 ± 0.8	1.59	31.5	31
cis-**20**	Thiazolidine	Ester			3.5 ± 0.6	2.86	17.5	
17	Thiophene	Ester	72 ± 4	> 90	0.89 ± 0.15	11.2	4.45	39
12	Triazole	Amide	< 10	22 ± 3	35 ± 5	0.29	175	26
18	Thiophene	Amide	28 ± 2	65 ± 5	5.1 ± 0.9	1.96	25.5	35
23	Triazole	Amino-oxetane	20 ± 3	58 ± 4	7.8 ± 1.2	1.28	39.0	35
24	Thiophene	Amino-oxetane	65 ± 3	> 90	0.99 ± 0.14	10.1	4.95	44

[a] Data are the means of measurements in three technical replicates. [b] [Inhib] = 10 µM, [TPP] = 50 µM. [c] [Inhib] = 10 µM, [TPP] = 10 µM. [d] IC_{50} values determined at [TPP] = 10 µM (refer to Figure S7). [e] Affinity of the inhibitor versus that of TPP, *i.e.* [TPP]/IC_{50} (= $K_{M(TPP)}/K_I$). [f] K_I is based on the previously reported K_M of TPP = 50 nM.[2,16] [g] Taken from Ref. 21.

To gain insights into the binding mode from *in silico* studies, **24** was docked into the active site of human PDH E1 and it overlayed well with TPP (Figure 2a,b), consistent with its TPP-competitive nature. Unlike TPP which uses its pyrophosphate to form ionic contacts with a Mg^{2+} ion, **24** has its aromatic amino-oxetane tail occupying the pyrophosphate pocket with the oxygen atom of the oxetane coordinated to the Mg^{2+} and the methoxy group hydrogen bonded to the backbone amide of Ala88. The predicted binding interactions of **24** in the coenzyme pocket are shown in Figure 2c: the aminopyrimidine-CH_2-thiophene portion of **24** makes the same interactions with the enzyme as does the thiamine portion of TPP. In amides the carbon and oxygen atoms are 1.2 Å apart, whereas in the 3-amino-oxetane isostere the equivalent atoms are 2.2 Å apart.[31] This greater separation allows the O atom of the oxetane to coordinate to the Mg^{2+} ion. The greater affinity of the amino-oxetane series (Table 1) can be attributed both to this coordination and to the higher hydrophobicity of the oxetane (which would reduce the desolvation penalty upon binding). The selectivity of **24** for PDH E1 over other TPP-dependent enzymes seems to be due to steric reasons; the other enzymes have smaller pyrophosphate pockets, which cannot accommodate the bulky tail.[19] In support of this, compounds **23** and **24** were docked into the active sites of each of the four other TPP-dependent enzymes and, in addition, **11** and **18** were docked into *Sc*PDC and *Dr*DXPS. In no case did the compound show a docking pose with the aminopyrimidine ring in the aminopyrimidine pocket *and* the other terminal ring in the pyrophosphate pocket; in all cases only one of these two pockets was occupied and the other ring was in the entrance of the active site (Figures S3-6).

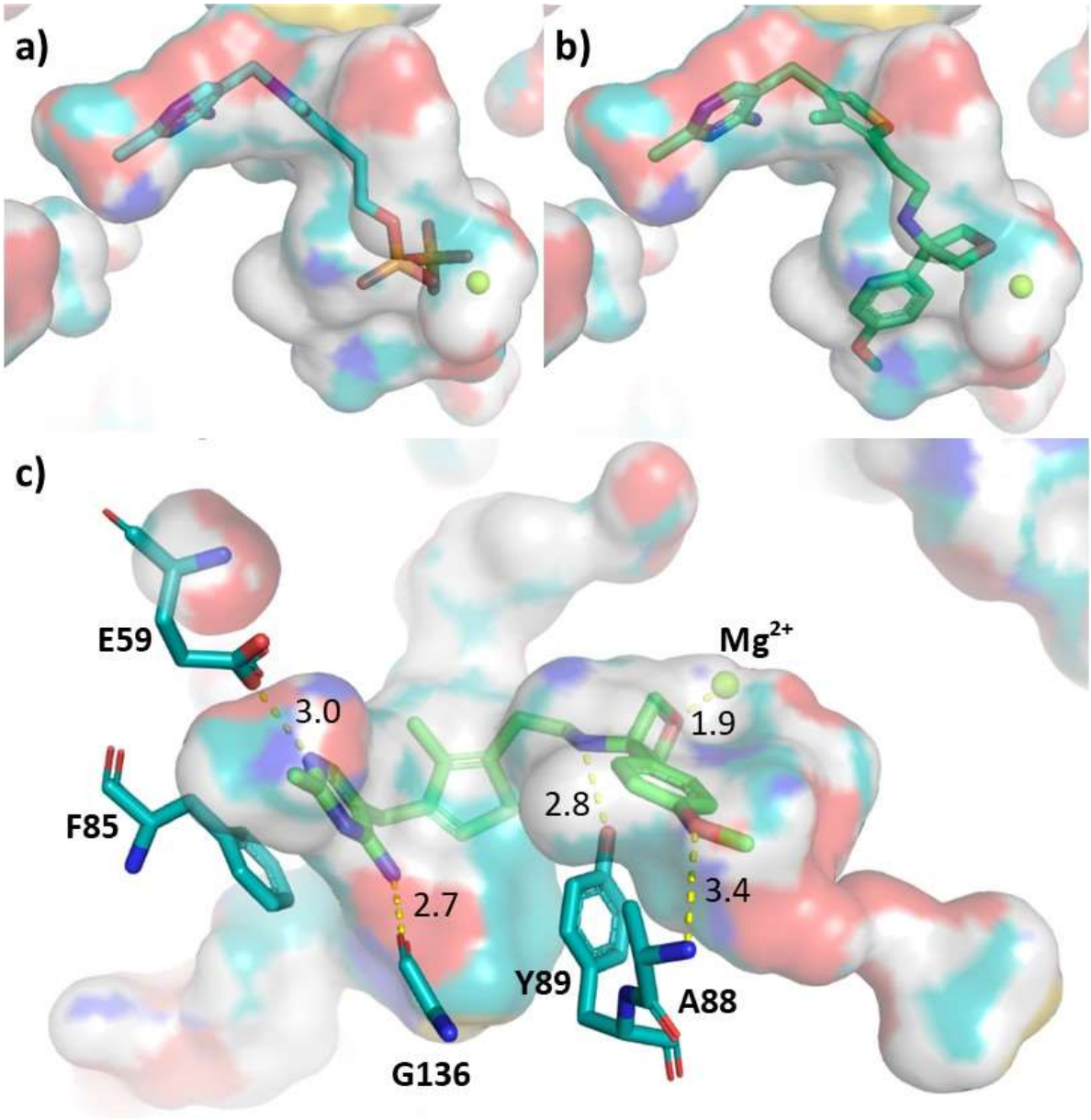


Figure 2. a) Binding mode of TPP (shown in stick mode) in human PDH E1 (PDB: 6CFO, surface of active site cavity shown) showing the V-shaped conformation between aminopyrimidine (left) and the thiazolium ring (right). **b)** Predicted binding mode of **24** (green atoms) as in view (a); **24** overlays well with TPP. **c)** Interactions between **24** and PDH E1 binding pocket shown as surface representation with interactions and distances shown as dashed lines. Mg^{2+} is represented as a yellow-green sphere.

TPP analogues (such as deazaTPP, the pyrophosphorylated form of **13**), as with other diphosph(on)ate compounds, are polyanionic under physiological conditions, and so are membrane-impermeable and cannot be used in cell-based assays.[16,32,33] This has long been a barrier in applying TPP analogues to study the cellular roles of vitamin B1. To show the compounds studied here are permeable, selected compounds were subjected to Parallel Artificial Membrane Permeability Assay (PAMPA).[34] As shown in Table 1 (refer to Table S2 for details), all tested compounds were, to some extent, membrane-permeable (fraction absorbed = 26-44%), but **24** was the most permeable.

Oxetane **24** is more membrane-permeable and more potent as a PDH E1 inhibitor than **18** but similar to its ester equivalent **17**. Thus, the stability of **17** and **24** under various reaction conditions was

investigated, based on compound recovery (assessed by ^{1}H NMR with 1,4-dinitrobenzene as internal standard) after various challenges (Table 3). Stirring at 37 °C in 0.1 M aq. phosphate buffer (pH 7.4) and heating at 70 °C in DMSO for 2 h resulted in excellent recovery for both compounds. Amino-oxetane **24** displayed excellent stability towards hydrolysis under basic conditions, but significant compound loss was observed with ester **17** in 1 M NaOH at 37 °C for 2 h or even 0.1 M K_2CO_3 at room temperature for 1 h. Both compounds were unstable under 1 M HCl at 37 °C for 2 h, with ester **17** showing better recovery. We speculate that the amino group of **24** would be protonated under acidic conditions, and the electron-donating methoxy group may facilitate the departure of the amino portion by stabilising the resultant cation.[35]

Given that esterase activity is ubiquitous in mammalian cells and plays a primary role in metabolising ester-containing drugs,[36] **24** was subjected to this biochemical challenge. Ester **17** was fully degraded after treatment with pig liver esterase at 37 °C and pH 7.4 for 30 min, but amino-oxetane **24** was almost completely recovered (Table 3). UPLC/MS[36] showed that esterase treatment for 4 hours at 37 °C and pH 7.4 completely hydrolysed ester **17** to alcohol **13**, while amide **18** and amino-oxetane **24** remained stable (Figure S5).

Table 3. Summary on stability based on compound recovery determined by proton NMR.

Reaction conditions	Compound recovery (%)	
	Ester **17**	Amino-oxetane **24**
0.1 M aq. phosphate buffer (pH 7.4), 37 °C, 2 h	> 90	> 90
DMSO, 70 °C, 2 h	> 90	> 90
1 M aq. NaOH, 37 °C, 2 h	15	> 90
0.1 M aq. K_2CO_3, rt, 1 h	31	> 90
1 M aq. HCl, 37 °C, 2 h	43	22
Porcine esterase, 37 °C, 30 min	< 10	> 90

In conclusion, as shown here and previously,[19] thiamine analogues featuring an ester-linked tail to bind in the pyrophosphate pocket are potent and selective inhibitors of PDH E1. However, changing to an amide linker, which improved stability, weakened binding. Here, the amino-oxetane isosteres of the amides were efficiently prepared by simply changing the amine's coupling partner from *p*-anisic acid to reagent **22**.[23] The resulting amino-oxetane **24** was resistant to esterase hydrolysis and a selective inhibitor of PDH E1 (over other TPP-dependent enzymes), and both more potent and more membrane-permeable than the corresponding amide **18**. Using the terminal *p*-methoxy group (in place of the potentially problematic *m*-pyridin-3-yl substituent) and the central thiophene ring (the strongest scaffold to date[21]), amino-oxetane **24** was 2.5-fold more potent than amide **8** (hitherto the most potent esterase-stable inhibitor). As the longest linear route to **24** consisted of nine steps from 2,3,5-tribromo-4-methylthiophene,[21] **24** is more accessible than **8** (twelve steps needed[19]). We envision that thiamine analogue **24** will aid work using chemical inhibition to understand the oncogenic role of PDHc. We hope this study will also serve to promote the use of amino-oxetanes as amide isosteres in drug discovery campaigns.

Acknowledgements

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Author Contributions

AHYC synthesised all compounds and conducted stability tests. TCSH performed all enzyme assays and computational dockings. AHYC and TCSH together conducted PAMPA and the esterase assay. FJL supervised the project. All authors approved the final version of the paper.

Conflicts of Interest

The authors declare there are no competing financial interest.

Supplementary Data

Supplementary data to this article can be found online at : Methods and results for enzyme assays and computational docking; synthetic methods, compound characterisation and NMR spectra.

References

1 Frank RAW, Leeper FJ, Luisi BF. Structure, mechanism and catalytic duality of thiamine-dependent enzymes. *Cell. Mol. Life Sci.* 2007, **64**, 892-905. https://doi.org/10.1007/s00018-007-6423-5

2 Walsh DA, Cooper RH, Denton RM, Bridges BJ, Randle PJ. The elementary reactions of the pig heart pyruvate dehydrogenase complex. A study of the inhibition by phosphorylation. *Biochem. J.* 1976, **157**, 41-67. https://doi.org/10.1042/bj1570041

3 Prajapati S, Rabe von Pappenheim F, Tittmann K. Frontiers in the enzymology of thiamin diphosphate-dependent enzymes. *Curr. Opin. Struct. Biol.* 2022, **76**, 102441. https://doi.org/10.1016/j.sbi.2022.102441

4 Park S, Jeon J-H, Min B-K, Ha C-M, Thoudam T, Park B-Y, Lee I-K. Role of the pyruvate dehydrogenase complex in metabolic remodeling: differential pyruvate dehydrogenase complex functions in metabolism. *Diabetes Metab. J.* 2018, **42**, 270-281. https://doi.org/10.4093/dmj.2018.0101

5 Liberti MV, Locasale JW. The Warburg effect: how does it benefit cancer cells? *Trends Biochem. Sci.* 2016, **41**, 211-218. https://doi.org/10.1016/j.tibs.2015.12.001

6 Sutendra G, Michelakis ED. Pyruvate dehydrogenase kinase as a novel therapeutic target in oncology. *Front. Oncol.* 2013, **3**, 38. https://doi.org/10.3389/fonc.2013.00038

7 Hensley CT, Faubert B, Yuan Q, Lev-Cohain N, Jin E, Kim J, Jiang L, Ko B, Skelton R, Loudat L, Wodzak M, Klimko C, McMillan E, Butt Y, Ni M, Oliver D, Torrealba J, Malloy CR, Kernstine K, Lenkinski RE, DeBerardinis RJ. Metabolic heterogeneity in human lung tumors. *Cell* 2016, **164**, 681-694. https://doi.org/10.1016/j.cell.2015.12.034

8 Davidson SM, Papagiannakopoulos T, Olenchock BA, Heyman JE, Keibler MA, Luengo A, Bauer MR, Jha A.K, O'Brien JP, Pierce KA, Gui DY, Sullivan LB, Wasylenko TM, Subbaraj L, Chin CR, Stephanopolous G, Mott BT, Jacks T, Clish CB, Vander Heiden MG, Environment impacts the metabolic dependencies of Ras-driven non-small cell lung cancer. *Cell Metab.* 2016, **23**, 517-528. https://doi.org/10.1016/j.cmet.2016.01.007

9 Chen J, Guccini I, Di Mitri D, Brina D, Revandkar A, Sarti M, Pasquini E, Alajati A, Pinton S, Losa M, Civenni G, Catapano CV, Sgrignani J, Cavalli A, D'Antuono R, Asara JM, Morandi A, Chiarugi P, Crotti S, Agostini M, Montopoli M, Masgras I, Rasola A, Garcia-Escudero R, Delaleu N, Rinaldi A,

Bertoni F, de Bono J, Carracedo A, Alimonti A. Compartmentalized activities of the pyruvate dehydrogenase complex sustain lipogenesis in prostate cancer. *Nat. Genet.* 2018, **50**, 219-228. https://doi.org/10.1038/s41588-017-0026-3

10 Mann S, Perez Melero C, Hawksley D, Leeper FJ. Inhibition of thiamin diphosphate dependent enzymes by 3-deazathiamin diphosphate. *Org. Biomol. Chem.* 2004, **2**, 1732-1741. https://doi.org/10.1039/B403619K

11 Erixon KM, Dabalos CL Leeper FJ, Synthesis and biological evaluation of pyrophosphate mimics of thiamine pyrophosphate based on a triazole scaffold. *Org. Biomol. Chem.* 2008, **6**, 3561-3572. https://doi.org/10.1039/B806580B

12 Thomas AA, De Meese J, Le Huerou Y, Boyd SA, Romoff TT, Gonzales SS, Gunawardana I, Kaplan T, Sullivan F, Condroski K, Lyssikatos JP, Aicher TD, Ballard J, Bernat B, DeWolf W, Han M, Lemieux C, Smith D, Weiler S, Wright SK, Vigers G, Brandhuber B. Non-charged thiamine analogs as inhibitors of enzyme transketolase. *Bioorg. Med. Chem. Lett.* 2008, **18**, 509-512. https://doi.org/10.1016/j.bmcl.2007.11.098

13 Iqbal A, Sahraoui E-H, Leeper FJ, Gold(I)-catalysed synthesis of a furan analogue of thiamine pyrophosphate. *Beilstein J. Org. Chem.* 2014, **10**, 2580–2585. https://doi.org/10.3762/bjoc.10.270

14 Feng J, He H, Zhou Y, Cai M, Peng H, Liu H, Liu L, Feng L, He H. Structure optimization and bioactivity evaluation of ThDP analogs targeting cyanobacterial pyruvate dehydrogenase E1. *Bioorg. Med. Chem.* 2019, **27**, 115159. https://doi.org/10.1016/j.bmc.2019.115159

15 Chan XWA, Wrenger C, Stahl K, Bergmann B, Winterberg M, Müller IB, Saliba KJ. Chemical and genetic validation of thiamine utilization as an antimalarial drug target. *Nat. Commun.* 2013, **4**, 2060. https://doi.org/10.1038/ncomms3060

16 Grabowska E, Czerniecka M, Czyżewska U, Zambrzycka A, Łotowski Z, Tylicki A. Differences in the efficiency of 3-deazathiamine and oxythiamine pyrophosphates as inhibitors of pyruvate dehydrogenase complex and growth of HeLa cells in vitro. *J. Enz. Inhib. Med. Chem.* 2021, **36**, 122–129. https://doi.org/10.1080/14756366.2020.1844681

17 Chan AHY, Fathoni I, Ho TCS, Saliba KJ, Leeper FJ. Thiamine analogues as inhibitors of pyruvate dehydrogenase and discovery of a thiamine analogue with non-thiamine related antiplasmodial activity. *RSC Med. Chem.* 2022, **13**, 817-821. https://doi.org/10.1039/D2MD00085G

18 Chan AHY, Ho TCS, Agyei-Owusu K, Leeper FJ. Synthesis of pyrrothiamine, a novel thiamine analogue, and evaluation of derivatives as potent and selective inhibitors of pyruvate dehydrogenase *Org. Biomol. Chem.* 2022, **20**, 8855–8858. https://doi.org/10.1039/d2ob01819e

19 Chan AHY, Ho TCS, Parle DR, Leeper FJ. Furan-based inhibitors of pyruvate dehydrogenase: SAR study, biochemical evaluation and computational analysis. *Org. Biomol. Chem.* 2023, **21**, 1755-1763. https://doi.org/10.1039/D2OB02272A

20 Chan AHY, Ho TCS, Fathoni I, Pope R, Saliba KJ, Leeper FJ. Inhibition of thiamine diphosphate-dependent enzymes by triazole-based thiamine analogues. *ACS Med. Chem. Lett.* 2023, **14**, 621–628. https://doi.org/10.1021/acsmedchemlett.3c00047

21 Chan AHY, Ho TCS, Irfan R, Hamid RAA, Rudge ES, Iqbal A, Turner A, Hirsch AKH, Leeper FJ Design of thiamine analogues for inhibition of thiamine diphosphate (ThDP)-dependent enzymes: Systematic investigation through scaffold-hopping and C2-functionalisation. *Bioorg. Chem.* 2023, **138**, 106602. https://doi.org/10.1016/j.bioorg.2023.106602

22 Subbaiah MAM, Meanwell NA. Bioisosteres of the phenyl ring: recent strategic applications in lead optimization and drug design. *J. Med. Chem.* 2021, **64**, 14046-14128. https://doi.org/10.1021/acs.jmedchem.1c01215

23 Rojas JJ, Croft RA, Sterling AJ, Briggs EL, Antermite D, Schmitt DC, Blagojevic L, Haycock P, White AJP, Duarte F, Choi C, Mousseau JJ, Bull JA. Amino-oxetanes as amide isosteres by an alternative defluorosulfonylative coupling of sulfonyl fluorides. *Nat. Chem.* 2022, **14**, 160–169. https://doi.org/10.1038/s41557-021-00856-2

24 Meanwell NA. Improving drug candidates by design: a focus on physicochemical properties as a means of improving compound disposition and safety. *Chem. Res. Toxicol.* 2011, **24**, 1420–1456. https://doi.org/10.1021/tx200211v

25 Hopkins AL, Keserü GM, Leeson PD, Rees DC, Reynolds CH. The role of ligand efficiency metrics in drug discovery. *Nat. Rev. Drug Discov.* 2014, **13**, 105–121. https://doi.org/10.1038/nrd4163

26 He H, He H, Feng L, Peng H, Tan X. Compound as well as preparation method thereof and applications. Chinese patent CN106588887A, 2017.

27 Clarke GM, Sykes P. The reduction of thiazolium salts with sodium borohydride. Part II. The mechanism and stereochemistry of reduction. *J. Chem. Soc. C*, 1967, 1411. https://doi.org/10.1039/J39670001411

28 Kumari S, Carmona AV, Tiwari AK, Trippier PC. Amide bond bioisosteres: strategies, synthesis, and successes. *J. Med. Chem.* 2020, **63**, 12290–12358. https://doi.org/10.1021/acs.jmedchem.0c00530

29 Hamzik PJ, Brubaker JD, Reactions of oxetan-3-tert-butylsulfinimine for the preparation of substituted 3-aminooxetanes. *Org. Lett.*, 2010, **12** 1116–1119. https://doi.org/10.1021/ol100119e

29 Lai Z, Zhang R, Fenga Q, Sun J. 3-Aminooxetanes: versatile 1,3-amphoteric molecules for intermolecular annulation reactions. *Chem. Sci.*, 2020, **11**, 9945-9949. https://doi.org/10.1039/d0sc04254d

30 Wermuth CG, Optical Isomerism in Drugs, Chap. 18 in "The Practice of Medicinal Chemistry" (4th Ed.), eds Wermuth CG, Aldous D, Raboisson P, Rognan D, Academic Press, 2015. https://doi.org/10.1016/B978-0-12-417205-0.00018-3

31 Bull JA, Croft RA, Davis OA, Doran R, Morgan KF. Oxetanes: recent advances in synthesis, reactivity, and medicinal chemistry. *Chem. Rev*. 2016, **116**, 12150−12233. https://doi.org/10.1021/acs.chemrev.6b00274

32 Rudge ES, Chan AHY, Leeper FJ. Prodrugs of pyrophosphates and bisphosphonates: disguising phosphorus oxyanions. *RSC Med. Chem.*, 2022, **13**, 375–391. https://doi.org/10.1039/D1MD00297J

33 Hecker SJ, Erion MD. Prodrugs of phosphates and phosphonates. *J. Med. Chem.*, 2008, **51**, 2328–2345. https://doi.org/10.1021/jm701260b

34 Avdeef A. The rise of PAMPA. *Expert Opin. Drug Metab. Toxicol.*, 2005, **1**, 325–342. https://doi.org/10.1517/17425255.1.2.325

35 Saejong P, Rojas JJ, Denis C, White AJP, Voisin-Chiret AS, Choi C, Bull JA. Synthesis of oxetane and azetidine ethers as ester isosteres by Brønsted acid catalysed alkylation of alcohols with 3-aryl-oxetanols and 3-aryl-azetidinols. *Org. Biomol. Chem.*, 2023, 21, 5553-5559. https://doi.org/10.1039/D3OB00731F

36 Perez C, Daniel KB, Cohen SM. Evaluating prodrug strategies for esterase-triggered release of alcohols. *ChemMedChem*, 2013, **8**, 1662–1667. https://doi.org/10.1002/cmdc.201300255